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Short Communication

Bioelectrochemical approach for enhancing lignocellulose degradation and biofilm formation in *Geobacillus* strain WSUCF1

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ABSTRACT

Investigations on microbial electrocatalysis as a strategy for enhancing the rates of substrate utilization leading to enhanced yield of biomass and enhanced biofilm formation are reported. A thermophilic *Geobacillus* sp. strain WSUCF1 (60 °C), a potential lignocellulose degrading microorganism was used as the electrocatalyst. Glucose, cellulose, and corn stover were used as the feedstocks. The results of this investigation showed that applying the oxidation potential of −0.383 mV (vs PRE) increased the glucose utilization and COD removal by 25.5% and 29.7% respectively. The bioelectrocatalysis strategy also increased the biomass yield by 81.2, 42.1, and 49.5% in the case of systems fed with glucose, cellulose, and corn stover, respectively, when compared with the systems without applied oxidation potential. This is the first work reporting the effects of applied oxidation potential on increasing the rates of degradation of lignocellulosic biomass and enhanced biofilm formation.

1. Introduction:

Electroactive microorganisms are becoming increasingly popular in recent decades, owing to their wide range of applications including production of biofuels such as bioelectricity (Shrestha et al., 2018), biohydrogen (Pasupuleti et al., 2015), biomethane (Baek et al., 2017), and biohythane (Liu et al., 2016); and bioremediation of effluents and recalcitrant waste (Rathinam et al., 2013a,b; Selvaraj et al., 2016), and production of value-added compounds (Rathinam et al., 2014). Electroactive microorganisms have an additional capability to mediate electron transfer reactions at electrode-electrolyte interfaces using direct or mediated electron transfer mechanisms (Rathinam et al., 2018a,b,c,d). Electroactive microorganisms have shown to be promising for utilizing a wide range of substrates such as glucose, ethanol, acetate, domestic wastewater and other industrial effluents (Velvizhi et al., 2017). However, use of lignocellulosic feedstocks in bioelectrochemical systems have several limitations due to their recalcitrant nature.

Different strategies such as use of cocultures and consortia were documented in the literature for enhanced utilization of lignocellulosic feedstocks (Rathinam et al., 2014). Rathinam et al. (2015) reported a three chambered microbial fuel cell coupling the cellulolytic activity of *Oscillatoria annae* and electrogenic activity of cocultures of *Acetobacter aceti* and *Glucanovacter roseus*. Bhuvaneswari et al. (2013) reported the bioelectricity production using *Staphylococcus aureus* isolated from rumen fluid with cellulose as a sole source of carbon. Reports are also documented on the use of lignocellulosic biomass in microbial desalination cells and microbial electrolysis cells (Nam et al., 2014). However, in most of cases, the electroactive microorganisms are used for converting the chemical energy to electrical energy. The low power output remains a major limitation that hinders the industrial applications of this process. On the other hand, investigations on harnessing the concept of microbial electrocatalysis for enhancing the rates of lignocellulose degradation, and production of biomass and value-added products are scarce. This strategy would be advantageous in accelerating the utilization rates of lignocellulosic materials and the production of value-added products such as biofuels, making it promising for practical applications.

Several reports are available in the literature on the bioelectrosynthesis of biofuels and other value-added compounds. Bioelectrosynthesis of acetate (May et al., 2016), methane (Zhen et al., 2015) and hydrogen (Kitching et al., 2017) were reported in the literature. Yu et al., 2017., reported the use of *Moorella thermoautotrophica,* a thermophilic bacterium as the electrocatalysts for the synthesis of formate and acetate. *Moorella thermoautotrophica* displayed temperature dependent electron uptake, and electrosynthesis rates of

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formate and acetate increased by 23.2 and 2.8-fold at 55 °C, respectively when compared to the experiment at 25 °C.

Herein, development of a microbial electrolytic process using *Geobacillus* sp. WSUCF1 for enhancing the rates of degradation of lignocellulosic-feedstocks is reported. *Geobacillus* sp. strain WSUCF1 is a cellulose degrading thermophilic strain isolated from a compost facility at Washington State University (WSU), Pullman, WA that can grow at an optimal temperature of 60 °C. *Geobacillus* sp. strain WSUCF1 was shown to retain 89% of its initial CMCase activity after incubation at 70 °C for 1 day (Rastogi et al., 2010). Cellulolytic activity of this strain is evident from its genome sequence encoding several genes for glycoside hydrolases (Bhalla et al., 2013). This strain was shown to produce highly thermostable β-xylosidase (when compared with other thermophiles reported in the literature) with high specific activity (133 U/mg when incubated with p-nitrophenyl xylopyranoside) (Bhalla et al., 2014). Electrogenic activity of a similar thermophilic strain, *Geobacillus* sp. strain 44-C, was previously documented in the literature and was shown to have redox proteins involved in electron transfer (Rathinam et al., 2018a,b,c,d). The present study harnesses both the lignocellulose-degrading capabilities and electrogenic activity of *Geobacillus* sp. WSUCF1. This facile strategy may help in the safe disposal of wastes without sophisticated conditions in an environmentally benign manner. The ease of scale up and inexpensive nature of this microbial electrocatalysis strategy makes this promising for practical applications.

2. Materials and methods

2.1. Growth of microorganisms

Geobacillus sp. strain WSUCF1 was used as electrocatalysts for electrocatalysis experiment. The cultures of *Geobacillus* sp. strain WSUCF1 was inoculated in Luria Bertani broth and incubated at 60 °C for 24 h. After incubation, the cells were obtained by centrifuging the broth containing the cells at 10000 rpm for 10 min. The cell debris and broth constitute were removed from the cell pellets by washing with sodium phosphate buffer (0.1 M, pH 7). The cells were then dispersed in the sodium phosphate buffer and used for the electrochemical investigations and electrosynthesis studies.

2.2. Fabrication of electrodes

Electrodes were fabricated using carbon felt procured from Fuel Cell Earth LLC, having area 1.0 cm^2. A brass rod was used to provide electrical contact. Cell pellet was dispersed in the phosphate buffer containing glucose and was kept at anaerobic conditions. Constant stirring at 10000 rpm was provided for biofilm formation. Biofilm was allowed to form until the electrode reached a stable negative potential. Biofilms formed onto fabricated electrodes were used for investigating the electrogenic activity and performing bioelectrosynthesis experiments (Rathinam et al., 2015). Sodium phosphate buffer solutions (0.1 M, pH 7) were used as electrolyte.

2.3. Electrochemical analysis

The electrogenic activity of the biofilm was analysed using cyclic voltammetry. Cyclic voltammograms (CV) of *Geobacillus* sp. WSUCF1 was recorded with the bioelectrodes in phosphate buffer (0.1 M, pH = 7). Silver wire and platinum (Pt) were used as a pseudo-reference and counter electrode, respectively. The electrochemical investigations were conducted at aseptic conditions, and the temperature was maintained at 60 °C. CV plots of *Geobacillus* sp. strain WSUCF1 were recorded at scan rate of 10 mV/s with glucose as the electron donor (Rathinam et al., 2013a,b).

2.4. Bioelectrosynthesis experiment

Bioelectrosynthesis experiments were conducted by applying a specific oxidation potential of the electron donor using long-term chronoamperometry as described previously (Shrestha et al., 2018; Rathinam et al., 2014). Experiments were conducted with three different feedstocks namely glucose, cellulose, and corn stover. The effect of oxidation potential on the glucose utilization, COD removal rate, biomass yield, and biofilm formation was investigated. Control experiments were conducted with respective electron donors and biofilm electrodes without applied oxidation potential. Electrolyte samples collected after the experiment were used for quantification of glucose and COD levels. Levels of glucose were estimated using the dinitrosalicilylic methods (Miller et al., 1959). COD digester (Spectroquant 320, Merck) was used for measuring the COD levels. Changes in the wet weight of the biomass in both bioelectrocatalysis and control experiments with three different substrates namely glucose, cellulose, and corn stover were also noted. The effect of applied oxidation potential on the biofilm formation was also investigated. *Geobacillus* sp. WSUCF1 biofilms formed on the carbon felt in control and electrosynthesis experiments were characterized using Scanning Electron Microscopy. Carbon felt containing the biofilm of dimension 0.5x0.5 cm was cut and dried in a desiccator under aseptic conditions. Biofilms formed onto the electrodes were analysed using a Zeiss Supra40 variable-pressure field-emission SEM.

3. Results and discussion

3.1. Electrogenic activity

Cyclic voltammograms of bioelectrodes were recorded from a potential range of −1.0 V to +1.0 V (vs PRE) at a scan rate of 5 mV/s and are shown in Fig. 1. CV of the bioelectrode displayed a redox peak at −0.383 mV (vs PRE) and the peak current increased with addition of 100 µL of 1 mM of glucose. Addition of 100 µL of 5 mM of glucose increased the anodic peak current from 0.0030 mA to 0.0050 mA. With subsequent addition of 5 mM of glucose (100 µL), the current increased to 0.0055 mA and 0.0060 mA respectively. These results confirm the electrocatalytic activity of *Geobacillus* sp. strain WSUCF1 towards glucose. This study clearly indicates that the peak at −0.383 mV (vs PRE) might be due to the key enzymes involved in glucose oxidations viz. glucose oxidation or glucose dehydrogenase. Wang et al., 2013 showed the CVs of glucose oxidase displayed onto yeast surface had a formal potential at −0.489 V. This potential corresponds to the redox site of the glucose oxidase ($FAD/FADH_2$ redox center) that is involved in direct electron transfer from this site to the electrode surface.

3.2. Substrate utilization

Three sets of electrosynthesis experiments were conducted with glucose, cellulose, and corn stover by applying a potential of −0.383 mV (vs PRE) for four days. The effect of the applied oxidation

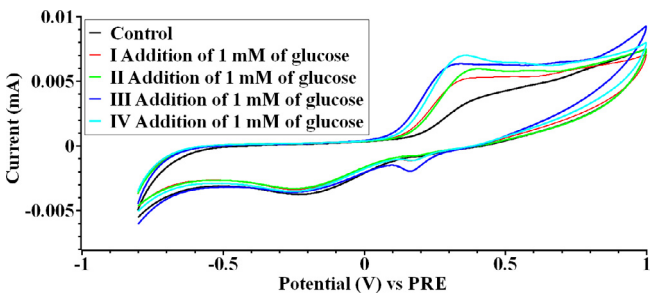

Fig. 1. Cyclic voltammograms of *Geobacillus* sp. strain WSUCF1 bioelectrodes on bioelectrocatalysis of glucose.

Table 1
Effect of applied oxidation potential on (A) substrate utilization (B) COD removal, and biomass yield in *Geobacillus* sp. strain WSUCF1.

	Substrates	Control	Electrosynthesis
Glucose utilization (μg/L)	Glucose	16.83 ± 0.21	21.13 ± 0.21
	Cellulose	17.06 ± 4.08	24.46 ± 1.21
	Corn stover	12.23 ± 3.00	24.03 ± 1.96
COD Removal (μg/L)	Glucose	57.33 ± 7.51	74.33 ± 5.86
	Cellulose	45.33 ± 8.39	51.67 ± 4.73
	Corn stover	55.67 ± 4.04	70.33 ± 4.04
Biomass growth (mg)	Glucose	0.88 ± 0.065	1.6 ± 0.068
	Cellulose	0.75 ± 0.019	1.12 ± 0.01
	Corn stover	0.82 ± 0.10	1.23 ± 0.08

potential on the substrate utilization in microorganisms was investigated by glucose utilization rate and COD removal rate. Samples were taken before and after the electrosynthesis experiment. Results of this investigation showed that on applying the oxidation potential of glucose, the glucose utilization rate increased by 25.5%, 43.3%, and 96.4% with glucose, cellulose, and corn stover as feed stocks respectively (shown in Table 1).

COD values measure the amount of substrate as well as the metabolites released in the oxidation processes via microbial electrocatalysis. Further investigations of the COD levels were carried out to consider the metabolites produced by the microorganisms which could correlate with the rates of metabolism in microorganisms with and without applying the oxidation potential. COD levels increased 29.7, 14.0, and 26.4% on applying the oxidation potential in the case of glucose, cellulose, and corn stover fed systems, respectively when compared with the control. The results of COD utilization rates with applied oxidation potential corroborate well with the glucose utilization rates. These results clearly indicate that this approach is promising for treatment of solid and liquid wastes with high COD levels. Table 1 depicts the effect of the applied oxidation potential on COD removal.

Applying the oxidation potential also increased the yield of biomass from 0.88 mg, 0.79 mg, and 0.82 mg in case of systems fed by glucose, cellulose, and corn stover without applied oxidation potential to 1.6, 1.1, and 1.2 mg in case of systems fed glucose, cellulose, and corn stover fed systems with applied oxidation potential (shown in Table 1). Biomass yield increased by 81.2, 42.1, and 49.5% with applied oxidation potential in the case of systems fed glucose, cellulose, and corn stover, respectively when compared with the systems without applied oxidation potential.

3.3. Biofilm formation

SEM images of carbon felt electrodes with biofilms of *Geobacillus* sp. WSUCF1 after the bioelectrocatalytic process and in the control (without applied external potential) were analysed to investigate the effect of applied oxidation potential on biofilm formation. Applying the oxidation potential mediates the microbial electrocatalysis, leading to enhanced oxidation rates. However, the electroactive microorganism transfers the electrons to an electron acceptor to create the proton gradient for ATP synthesis that is crucial for its survival. SEM images of the bioelectrodes with applied oxidation potential showed denser and thicker biofilm formation. The control electrodes also had biofilm formation but it was not as dense as compared with the one treated with external voltage. These results clearly indicate that the applied potential drives biofilm formation. Applying the oxidation potential promotes the adherence, growth, and proliferation of *Geobacillus* sp. WSUCF1 biofilms on the electrode surface leading to improved biofilm quality. Pinto et al., 2018, reported the effect of anode polarization on the *Shewanella oneidensis* biofilm formation on graphite felt electrodes and electron transfer characteristics. Applying a positive potential of 0.3 V (vs. Ag/AgCl/KCl sat.) favoured direct electron transfer whereas applying the negative potential of −0.3 V (vs. Ag/AgCl/KCl sat.) offered mediated electron transfer.

4. Conclusion

This study demonstrated for the first time electrogenic activity of a thermophile, *Geobacillus* sp. strain WSUCF1, that displayed electrocatalysis of glucose. The study showed that this bioelectrosynthesis strategy increased the substrate utilization significantly, leading to enhanced biomass yield. This approach will be promising for enhancing the yield of bioprocesses for industrial biotechnology applications. Further attempts to make a good choice of electrode materials, electrode functionalization strategies, and configurations of bioelectrochemical systems, will aid in improving the performance of biodegradation of wastes, including recalcitrant waste, and production of value-added compounds.

5. Declarations of interest

None.

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